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Absence of *Nsi*I Polymorphism in Growth Hormone Receptor (*GHR*) Gene in Indian Cattle Breeds

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ABSTRACT

The objective of the present study was to investigate the polymorphism in 5′ non-coding region of growth hormone receptor (*GHR*) gene in Sahiwal (n = 53) and Hariana (n = 50) cattle using *Nsi*I/PCR-RFLP assay. Amplification of DNA sample revealed 302bp product using specific primer pairs and digested by using *Nsi*I restriction enzyme. All the screened animals were found monomorphic in nature for *GHR*/*Nsi*I polymorphism. It revealed that only one type of uncut banding pattern (AA genotype); which was of 302bp. We could not identify any animal with GG and AG genotypes. Consequently, we could not perform association studies with milk production traits.

Keywords: Sahiwal, Hariana, PCR-RFLP, *GHR*, *Nsi*I Polymorphism, Milk production traits

Growth hormone plays an important role in the regulation of mammogenesis and lactation in farm animal. Growth hormone actions on target cells depend on the growth hormone receptor. It mediates the biological actions of growth hormone on target cells by transducing the signal across the cell membrane (Lincoln *et al*., 1995). Any nucleotide variation in the growth hormone (*GH*) and growth hormone receptor (*GHR*) genes might directly or indirectly affect milk production traits. Therefore, *GH* and *GHR* genes are potential candidate markers for selection purposes in cattle (Parmentier *et al*., 1999). Several single nucleotide polymorphisms (SNPs)/mutations in *GHR* gene and their association with milk production traits have been reported in the bovine *GHR* gene (Maj *et al*., 2004; Olenski *et al*., 2010; Akad *et al*., 2012). A SNP (A/G, transition) at *GHR* locus has also been reported at position -154 in 5′ non-coding region (Maj *et al*., 2004). Recently this polymorphism has been studied in various exotic and Indian cattle breeds including Hariana (Deepika and Salar, 2013). Till date no GHR/*Nsi*I polymorphism study has been studied in Sahiwal cattle. So therefore, the present study was undertaken to investigate the status of *GHR* gene polymorphisms (A/G) in Indian Sahiwal and Hariana cattle breeds using *Nsi*I/PCR-RFLP assay.

MATERIALS AND METHODS

Animal source and DNA Extraction

A total of 103 females of Sahiwal (n = 53) and Hariana (n = 50) cattle maintained at Instructional Livestock Farm complex (ILFC), DUVASU, Mathuta (Uttar Pradesh), were utilized in the present investigation. Genomic DNA was isolated from venous blood using the standard protocol of Sambrook and Russel (1991). An amplicon of 302 bp consisting of *GHR* 5′ non-coding region was amplified using a specific primer pairs (‘F’: 5′- CTG GCG TAT GGT CTT TGT CA - 3′ and ‘R’: 5′- TGG TCT TGC TTG

CTT TCC TAT -3′; Deepika and Salar, 2013). The cycle conditions included an initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 58°C for 30 sec and extension at 72°C for 25 sec and a final extension at 72°C for 8 min. The PCR product was checked on 1.0% agarose gel electrophoresis in 1x TAE buffer after staining with ethidium bromide (EtBr) and visualized under UV light.

Genotyping of animal using NsiI/PCR-RFLP assay

The restriction digestion was carried out at 37ºC for 14-16 h in a total volume of 15µl containing 5.0 µl of PCR product, 1.5 µl of 10X RE buffer and 10 Units (1.0 µl) *Nsi*I enzyme (New England Biolabs). For genotyping analysis, digested products were checked on 1.5% agarose gel with StepUp 100 bp DNA ladder (Merck Bioscience) in 1x TAE buffer for 2-3 h at 5 V/cm. The fragments were visualized under UV light after staining with EtBr. The data was generated by estimating the frequency of different RFLP pattern. The allelic frequency and genotypic frequencies of *GHR* gene was estimated by standard procedure (Falconer and Mackay, 1996).

RESULTS AND DISCUSSION

The amplified product of *GHR* gene was 302 bp (Fig.1). In the present study, all the screened Sahiwal and Hariana cattle were found monomorphic in nature for *GHR*/*Nsi*I polymorphism. It revealed that only one type of uncut banding pattern (AA genotype); which was of 302 bp. We could not identify any animal with GG homozygote (167 and 135 bp) and AG (302, 167 and 135 bp); heterozygote genotypes (Fig. 1).

The genotypic frequency of AA genotype was 100% in screened animals for present investigation. Similar results were observed in Tharparkar cattle by Deepika and Salar (2013). In contrast, AG genotype was also detected in Hariana cattle (Deepika and Salar, 2013). However, Deepika and Salar (2013) detected 94.2% animals were of AA genotype, 5.6% were of AG genotype and 0.2% animals were of GG genotype in total screened Indian cattle breeds. In contrast, the genotypic frequency of AA genotype was range from 0.0% to 4.5% in Turkish cattle breeds (Akad *et al*., 2012). In present study, the AG and GG genotypes were found absent, however, Deepika and Salar (2013) found the frequency of AG and GG genotype was ranged from 0.0 to 15.2 and 0.0 to 2.1, respectively in various Indian cattle breeds. The result of present study was found almost similar to Deepika and Salar (2013) in Indian cattle breeds. In contrast, Akad *et al*. (2012) observed the frequency range of AG and GG genotype was 36.0 to 61.2% and 38.7% to 52.2%, respectively in screened Turkish cattle breeds.

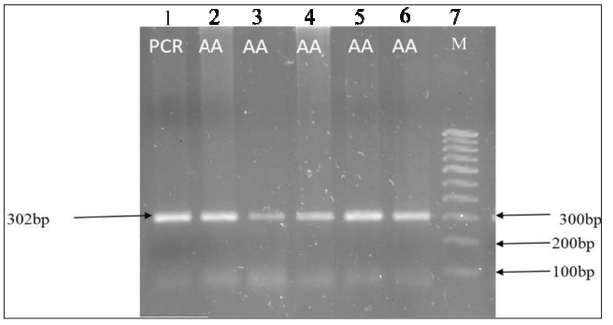

Fig. 1: GHR/*Nsi*I PCR-RFLP assay showing genotype pattern in 1.5% agarose gel; Lane1: Undigested PCR product (302 bp), (2-6): AA genotype (302 bp), 7: Marker (StepUp 100 bp DNA ladder, Cat No. MBD13).

The allelic frequency observed for allele A in the screened animal of Sahiwal and Hariana was 1.0 and for allele G was 0.0 in present work. Similar result was reported in Tharparkar but in Hariana cattle, frequency of G allele was 0.076 (Deepika and Salar, 2013). This variation may be due to different herd and selection history. The frequency distribution of A allele across the ten breeds ranged from 0.915 (Hill cattle) to 1.00 (Tharparkar) with a mean value of 0.970, whereas that of G allele ranged from 0.00 (Tharparkar) to 0.085 (Hill cattle) with a mean value of 0.030 (Deepika and Salar, 2013). In contrast, Akad *et al*. (2012) found allelic frequency range of A and G allele was 0.20 to 0.31 and 0.69 to 0.80, respectively in Turkish cattle breeds.

We could not perform association study with milk production traits in the present study, because all the screened animals were found monomorphic for GHR/*Nsi*I locus. However, previous studies reported that AA genotype of GHR/*Nsi*I locus significantly associated with more milk with higher concentration of milk components (Maj *et al*., 2004) in Polish Black and White cattle. The near fixation of favorable allele A due to significantly high

milk producing nature of these Indian cattle.

CONCLUSION

In present study, the status of *GHR* gene polymorphism was investigated using *Nsi*I/PCR-RFLP assay in Indian Sahiwal and Hariana cattle breeds and we report monomorphic pattern in the screened population. Consequently, we could not establish any association between genotypes and milk production trait because these cattle were found homozygous (AA) for *Nsi*I locus (A/G). It would be interesting to further investigate this SNP and its role in other Indian cattle breeds, therefore *GHR* could be used as potential genetic marker in MAS to improve the milk production performance in dairy cattle.

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